

2

eration and dispersal of air such that the L-lactate concentration of the product solution remains under 40 mmoles/l.

5,200,327

**EXPRESSION SYSTEM FOR THE SECRETION OF BIOACTIVE HUMAN GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) AND OTHER HETEROLOGOUS PROTEINS FROM STREPTOMYCES**

Robert T. Garvin, Toronto, and Lawrence T. Malek, Brampton, both of Canada, assignors to Cangene Corporation, Mississauga, Canada

Continuation-in-part of Ser. No. 795,331, Nov. 6, 1985, abandoned, and a continuation-in-part of Ser. No. 221,346, Jul. 18, 1988, abandoned. This application Jul. 26, 1988, Ser. No. 224,568

Claims priority, application Canada, Jul. 25, 1988, 572956

Int. Cl.<sup>3</sup> C12P 21/02; C12N 15/76, 15/00, 1/21

U.S. Cl. 435—69.5

21 Claims

1. A gene expression system comprising a regulatory nucleotide sequence operably linked to a nucleotide sequence encoding a heterologous protein, wherein

said regulator nucleotide sequence comprises a promoter sequence operably linked to a nucleotide sequence encoding a signal peptide;

said signal peptide is capable of directing the secretion of said heterologous protein in bioactive form from a host selected from the genus *Streptomyces*; and

said signal peptide is a hybrid of signal peptides of the genus *Streptomyces*.

7. A vector capable of transformation and replication in *Streptomyces* wherein said vector comprises a gene expression system of claim 1 or claim 5.

14. A process of producing a heterologous protein in a bioactive form that is secreted from a host selected from the genus *Streptomyces* comprising the steps of:

(A) transforming a host selected from the genus *Streptomyces* with a vector according to claim 7;

(B) growing a culture of the host produced by transformation with said vector under conditions such that said heterologous protein is expressed and secreted in said bioactive form; and

(C) recovering said heterologous protein from said culture.

5,200,328

**PROCESS FOR PRODUCING METHYL GLYCOSIDE ESTERS**

Ole Kirk, Copenhagen; Sven Erik Godtfredsen, Vaerloese, both of Denmark, and Fredrik Björklund, Helsingborg, Sweden, assignors to Novo Nordisk A/S, Bagsvaerd, Denmark

Filed Mar. 16, 1990, Ser. No. 494,702

Claims priority, application Denmark, Feb. 17, 1989, 0768/89 The portion of the term of this patent subsequent to Mar. 2, 2010, has been disclaimed.

Int. Cl.<sup>3</sup> C12P 19/04

U.S. Cl. 435—101

11 Claims

1. A process for preparing a compound of formula I

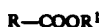


(I)

wherein

R is alkyl with 7-24 carbon atoms optionally substituted by hydroxy or halogen; and

X is a monosaccharide containing one hexose or pentose unit which carries (a) the  $-OCH_3$  group at the anomeric carbon atom and (b) the  $R-COO-$  group at a primary hydroxy group; comprising reacting (a) an acid or ester of formula II



(II)

wherein

R is as defined above; and

R<sup>1</sup> is H or lower alkyl; with (b) a glycoside of formula III



(III)

wherein

X is as defined above; in a substantially non-aqueous medium, in the substantial absence of a solvent other than the acid or ester of formula II acting as a solvent for the glycoside of formula III, and in the presence of an immobilized lipase.

5,200,329

**METHOD OF HYDROXYLATING**

**3-[(4,7-DICHLOROBENZOXAZOL-2-YL)METHYL]AMINO-5-ETHYL-6-METHYL-2-(1H)-PYRIDINONE BY INCUBATION WITH LIVER SLICES**

Suresh K. Balani, Hatfield; Anthony D. Theoharides, Lansdale, and Laura R. Kauffman, Jeffersonville, all of Pa., assignors to Merck & Co., Inc., Rahway, N.J.

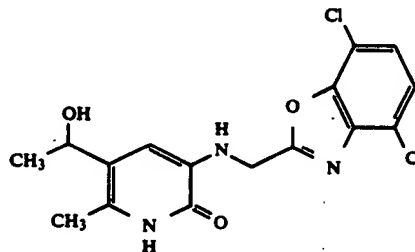
Continuation of Ser. No. 771,963, Oct. 4, 1991, abandoned. This application Mar. 10, 1992, Ser. No. 850,008

Int. Cl.<sup>3</sup> C12P 17/16

U.S. Cl. 435—118

2 Claims

1. A method of preparing the compound



or a pharmaceutically acceptable ester thereof, comprising the steps of

(1) providing a quantity of 3-[(4,7-dichlorobenzoxazol-2-yl)methyl]amino-5-ethyl-6-methyl-2-(1H)pyridinone,

(2) incubating the compound of step 1 with rat liver slices, and

(3) isolating the compound.

5,200,330

**METHOD FOR THE PREPARATION OF METHYL ANTHRANILATE**

Gregory V. Page, Maplewood; Bonita Scire, East Brunswick, and Mohamed I. Farbood, Holmdel, all of N.J., assignors to BASF K&F Corporation, Parsippany, N.J.

Division of Ser. No. 70,062, Jul. 6, 1987, abandoned. This application Sep. 14, 1990, Ser. No. 582,829

Int. Cl.<sup>3</sup> C12P 13/00, 13/02, 7/62

U.S. Cl. 435—128

8 Claims

1. A method for the production of methyl anthranilate comprising:

providing a microorganism selected from the group consisting of *Trametes versicolor* ATCC 42394 and *Polyporus* sp. ATCC 10089;

incubating said microorganism under aerobic conditions with substrate methyl N-methyl anthranilate in a nutrient broth for a period of time at a pH and at a temperature effective to allow said microorganism to convert said substrate to methyl anthranilate, wherein said pH is in a range of from about 3 to about 9, said temperature is in a range of from about 18° C. to about 33° C. and said incubation period is from 1 to 14 days; and recovering the methyl anthranilate.

tially pure R(+)-phenylethanol in the presence of NADPH, and wherein said dehydrogenase, in substantially pure form, (A) has an optimum pH of 7 for reduction of acetophenone and an optimum Ph of 8 for oxidation of phenylethanol; (B) has an optimum temperature of 25°-30° C.; (C) has a  $K_M$  value of  $6 \times 10^{-4}$  M for acetophenone; (D) has a  $K_M$  value of  $1.4 \times 10^{-4}$  M for NADPH; and (E) is rapidly inactivated by EDTA but is only weakly inhibited by inhibitors and chelators selected from the group consisting of 2,2'-dipyridine, 1,10-phenanthroline, iodoacetamide, p-hydroxymercuribenzoate, N-ethylmaleimide, phenylmethanesulfonyl fluoride and Triton X-100 and SH-protecting reagents selected from the group consisting of dithiothreitol and glutathione.

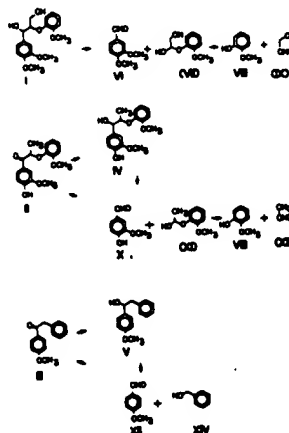
5,200,338  
**BACTERIAL EXTRACELLULAR LIGNIN PEROXIDASE**  
Donald L. Crawford, and Muralidhara Ramachandra, both of Moscow, Id., assignors to Idaho Research Foundation, Incorporation, Moscow, Id.

Filed Nov. 30, 1988, Ser. No. 277,802

Int. Cl.<sup>3</sup> C12N 9/24

U.S. Cl. 435—290

16 Claims



5,200,336  
**RESTRICTION ENDONUCLEASE OBTAINABLE FROM BACILLUS COAGULANS AND A PROCESS FOR PRODUCING THE SAME**

Huilin Kong, Beverly, and Ira Schildkraut, Hamilton, both of Mass., assignors to New England Biolabs, Inc., Beverly, Mass.

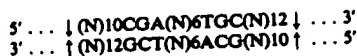
Filed Jul. 2, 1990, Ser. No. 547,787

Int. Cl.<sup>3</sup> C12N 9/22, 1/00

U.S. Cl. 435—199

6 Claims

1. A restriction endonuclease obtainable from *Bacillus coagulans*, ATCC No. 55055, where said endonuclease recognizes the following base sequence in double-stranded deoxyribonucleic acid molecules:



and cleaves said deoxyribonucleic acid molecules at both ends of the recognition sequence as indicated by the arrows.

5,200,337  
**NOVEL TYPE II RESTRICTION ENDONUCLEASE, APO I, OBTAINABLE FROM ARTHROBACTER PROTOPHORMIAE AND A PROCESS FOR PRODUCING THE SAME**

Carol Pollason, Arlington; Derek Robinson, Buxford, and Keith Lunnen, Newbury, all of Mass., assignors to New England Biolabs, Inc., Beverly, Mass.

Filed Oct. 25, 1991, Ser. No. 782,515

Int. Cl.<sup>3</sup> C12N 9/22

U.S. Cl. 435—199

12 Claims

1. A substantially pure Type II restriction endonuclease ApO I obtainable from *Arthrobacter protophormiae* (ATCC#55228) which recognizes and cleaves all permutations of the following base sequence in double-standard deoxyribonucleic acid molecules:



and having a cleavage defined by the arrows.

5,200,339  
**PROTEASES CAUSING ABNORMAL DEGRADATION OF AMYLOID  $\beta$ -PROTEIN PRECURSOR**

Carmela R. Abraham, 5 Blodgett Rd., Lexington, Mass. 02173

Continuation-in-part of Ser. No. 568,806, Aug. 17, 1990, abandoned. This application Apr. 5, 1991, Ser. No. 681,093

Int. Cl.<sup>3</sup> C12N 9/48; C12Q 1/37; A61K 37/547

U.S. Cl. 435—212

2 Claims

1. A brain-derived, calcium activated proteolytic factor comprising a serine protease that cleaves  $\beta$ -protein precursor at a site outside the  $\beta$ -protein domain and near the  $\beta$ -protein N-terminus, said serine protease migrating as a band having a molecular weight of about 30 kDa and a band having a molecular weight of about 68 kDa, as estimated by 12% SDS-PAGE using as molecular weight standards phosphorylase B, bovine serum albumin, ovalbumin and carbonic anhydrase.

5,200,340  
**THROMBIN-ACTIVATED TISSUE PLASMINOGEN ACTIVATORS**

Donald C. Foster; Eileen R. Mulvihill; Patrick J. O'Hara, all of Seattle, Wash.; Kurt Pingel, Farum, Denmark, and Shinji Yoshitake, Ibaraki, Japan, assignors to ZymoGenetics, Inc., Seattle, Wash.

Filed May 22, 1987, Ser. No. 53,412

Int. Cl.<sup>3</sup> C12N 9/61, 15/00, 15/58, 15/35

U.S. Cl. 424—94.64

11 Claims

1. A single chain form of a human t-PA, wherein said single chain form is cleavable by thrombin, said cleavage resulting in stimulation of amidolytic activity.

10) K-value:  
the K-value for the substrate N-acetyl-2,3-didehydroleucine  
is 4.5 mM (30° C., 0.1M glycine buffer, pH 9).

5,212,070

**SECRETORY SIGNAL SELECTION VECTORS FOR  
EXTRACELLULAR PROTEIN SYNTHESIS IN BACILLI**  
Hilde E. Smith, Groningen; Jan H. Van Es, Nieuwerkerk a/d  
IJssel; Ben P. H. Poeters, Haren; Sierd Bron, Haren, and  
Gerard Venema, Haren, Netherlands, assigns to Gist-  
brocades, Netherlands

Continuation of Ser. No. 45,890, May 1, 1987, Pat. No.  
5,037,760. This application Dec. 13, 1990, Ser. No. 627,028  
Int. Cl.<sup>3</sup> C12P 21/02, 19/34; C12N 15/00, 1/21; C07H 15/12;  
A61K 37/02

U.S. Cl. 435—69.1

4 Claims

1. In a method for producing a peptide product by recombinant techniques, wherein a host microorganism is transformed with a plasmid comprising a gene encoding said peptide product, and growing said host under nutrient conditions, whereby said gene is expressed and said peptide product is secreted, the improvement which comprises:

employing as said gene an open reading frame encoding said peptide product joined at its 5' terminus to a DNA sequence encoding an amino acid sequence capable of functioning as a secretory signal sequence (hereinafter "secretory sequence"), wherein said open reading frame DNA sequence is other than the native open reading frame of said DNA sequence encoding said secretory sequence and wherein said secretory sequence comprises one of the following amino acid sequences.

Met arg lys ser leu ile thr leu gly leu ala ser val  
ile gly thr ser ser phe leu ile pro phe thr ser lys  
thr ala ser ala glu thr leu asp glu lys lys glu lys  
ile glu ser lys glu ser glu val ala ser ser ile glu  
ala lys glu lys glu leu thr glu;

Met lys lys met leu val val leu leu phe ser ala leu leu  
leu am gly cys gly ser gly glu ser lys ala am thr ala  
glu thr pro glu val leu asp val lys leu thr gly;

Met ile arg gly ile leu ile ala val leu gly ile ala ile  
val gly;

Met leu lys lys val ile leu ala ala phe ile leu val gly  
ser;

Met ser glu glu his asp tyr val ile gly lys am ala val  
ile glu thr leu lys ser asp arg leu asp leu phe pro leu  
leu arg leu thr lys lys pro lys val glu thr gly ile asp  
thr leu leu pro asp tyr lys lys glu;

glu phe glu leu ala pro gly leu phe ile leu leu phe leu  
phe val met ala val ile gly;

Met leu lys arg thr ser phe val ser ser leu phe ile ser  
ser ala val leu leu ser ile leu leu pro ser gly leu ser  
his thr leu ser ala lys gly thr am lys am am leu phe  
phe phe asp thr glu thr thr gly leu gly gly ala gly  
am thr ile phe leu leu gly his ala arg val tyr glu asp  
arg val thr val lys glu his leu leu pro lys pro lys am  
glu val ala leu tyr glu ser phe leu ser glu val asp ile  
thr ser leu val thr tyr am gly lys ala phe asp tyr;

Met lys ile ser arg ile leu leu ala ala val ile leu ser  
ser val phe ser ile thr tyr leu glu ser asp leu gly thr  
phe ala lys glu gly glu met asp glu thr phe thr lys ala  
ala phe lys leu lys thr gly glu val ser asp;

Met lys glu thr val leu leu leu phe thr ala leu phe leu  
ser gly cys ser val ala ser ala asp asp ser val pro arg  
phe thr glu glu gly lys tyr ile gly ser ala asp;

Met lys lys leu val phe gly leu leu ala ile val leu phe  
gly cys gly leu tyr ile tyr his val trp phe gly asp;

-continued

Met leu lys lys cys ile leu leu val phe leu cys val gly  
leu ile gly leu ile gly cys ser lys thr asp ser pro glu  
asp;

Met arg lys trp ile ala ala ala gly leu ala tyr val leu  
tyr gly leu phe phe tyr trp tyr phe phe leu ser gly asp  
ser ala ile pro glu ala val lys gly thr glu ala asp;

Met pro ile lys lys lys val met met cys leu ala val thr  
leu val phe gly ser met ser phe pro thr leu thr am ser  
gly gly phe lys glu ser thr asp; and

Met lys leu val pro arg phe arg lys glu trp phe ala tyr  
leu thr val leu cys leu ala leu ala ala val ser phe  
gly val pro ala lys ala ala glu am pro glu thr ser val  
ser am thr gly lys glu ala asp ala thr lys am glu thr  
ser lys ala asp.

5,212,071

**NUCLEIC ACIDS ENCODING A HUMAN C3B/C4B  
RECEPTOR (CR1)**

Douglas T. Fearon, Baltimore, Md.; Lloyd B. Klickstein, Brookline, Mass.; Wianle W. Wong, Waban, Mass.; Gerald R. Carson, Wellesley, Mass.; Michael F. Conclao, Newton, Mass.; Stephen H. Ip, Sudbury, Mass., and Savvas C. Makrides, Bedford, Mass., assigns to The Johns Hopkins University, Baltimore, Md.; Brigham and Women's Hospital, Boston and T Cell Sciences, Inc., Cambridge, both of Mass.  
Continuation-in-part of Ser. No. 176,532, Apr. 1, 1988, abandoned. This application Apr. 3, 1989, Ser. No. 332,863  
Int. Cl.<sup>3</sup> C12N 15/12, 15/63

U.S. Cl. 435—69.1

64 Claims

1. An isolated nucleic acid encoding a polypeptide, the amino acid sequence of which comprises at least a fragment of the amino acid sequence depicted in FIG. 1, which polypeptide has a complement regulatory activity.

5,212,072

**POLYPEPTIDES COMPLEMENTARY TO PEPTIDES OR  
PROTEINS HAVING AN AMINO ACID SEQUENCE OR  
NUCLEOTIDE CODING SEQUENCE AT LEAST  
PARTIALLY KNOWN AND METHODS OF DESIGN  
THEREFOR**

J. Edwin Blalock; Kenneth L. Bost, both of Birmingham, Ala., and Eric M. Smith, Galveston, Tex., assigns to Board of Regents, The University of Texas System, Austin, Tex.  
Continuation of Ser. No. 829,709, Feb. 19, 1986, which is a continuation-in-part of Ser. No. 708,001, Mar. 1, 1983, Pat. No. 4,863,857. This application Mar. 7, 1991, Ser. No. 665,967  
Int. Cl.<sup>3</sup> C12P 21/06

U.S. Cl. 435—69.1

23 Claims

1. A polypeptide complementary to at least a portion of an original peptide or protein, said polypeptide being produced by a process comprising the steps of:

(a) determining a first nucleotide sequence of a first nucleic acid, said first nucleotide sequence coding for an amino

acid sequer  
or protein;  
(b) ascertaini  
nucleic aci  
sequence o  
nucleic aci

(c) determini  
tary poly  
coded by th  
same readi  
(d) producin  
sequence c

**PROCESS FOR  
Barrett Rollins  
Gordon G. V  
Genetics Inst  
FU**

Int. Cl.<sup>3</sup> C07H  
U.S. Cl. 435—

1. A DNA sequence set forth group consisting of:  
(i) the DNA  
(ii) a DNA c  
to the DN  
(iii) a DNA  
sequence  
2. A vector  
association wit  
3. A process  
(i) transform  
(ii) culturing  
conditions  
(iii) isolating

**GENETIC M  
GROWTH**

Michael C. K  
cisco, both c  
ville, Calif.  
Division of S  
appl.

Int. Cl.  
U.S. Cl. 435—

1. A recom  
coding insulin  
or a protein v

**COMPOSITI  
EFFEX**

Mark D. Bedn  
Jon O. Nag  
the Univers  
F

U.S. Cl. 435—  
1. A metho